HUNTINGDON LIFE SCIENCES LTD
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HUNTINGDON
CAMBRIDGESHIRE
PE18 6ES
ENGLAND

Report No.:

Title:

Study No.:

External Testing Facility No.:

Test Substance:

Study Director:

Sponsor:

Sponsor Representative:

Testing Facility: **Huntingdon Life Sciences Ltd.**
PO Box 2
Huntingdon
Cambridgeshire
PE18 6ES
ENGLAND

Study Completion Date: **10 February 2000**

Security Statement:

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ABSTRACT

The objective of this study was to assess the skin irritation potential of following a single dermal application to the rabbit.

The study was conducted using the EPA Health Effects Test Guidelines, OPPTS 870.2500 Acute Dermal Irritation EPA 712-C-98-196, August 1998.

was administered at a dose of 0.5 ml to one intact skin site on the clipped dorso-lumbar region of three female rabbits. Each treatment site was washed with warm water four hours after semi-occluded application of the test substance. The rabbits were scored for irritation at 56 minutes, 24, 48 and 72 hours following removal of the dressings, additional observations were made on Days 5 through 10 for all animals, on Day 11 for two animals and on Days 12 through 14 for one animal, following which the study was terminated.

There was no evidence of systemic response to treatment. A single semi-occlusive application of to intact rabbit skin for four hours elicited well-defined erythema in all three animals and was accompanied in one by very slight oedema. Desquamation of the stratum corneum developed in all animals. Dermal reactions had resolved completely in all animals by Day 10, 11 or 13.

The Primary Irritation Index (PII) was calculated to be 2.11.

GLP COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

United States Environmental Protection Agency, (TSCA), Title 40 Code of Federal Regulations Part 792, Federal Register, 29 November 1983 and subsequent amendment Federal Register 17 August, 1989.

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

The UK Good Laboratory Practice Regulations 1997 (Statutory Instrument No 654) and from 14 December 1999, the UK Good Laboratory Practice Regulations 1999 (Statutory Instrument No 3106).

EC Council Directive 87/18/EEC of 18 December 1986 (Official Journal No L 15/29), and from 1 May 1999 EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No L 77/8).

The raw data has been reviewed by the Study Director, who certifies that the information contained in this report is consistent with and supported by the raw data.

10 Feb 2000

Date

Study Director,
Huntingdon Life Sciences Ltd.

QUALITY ASSURANCE STATEMENT

Study Title: Skin Irritation of in the Rabbit

Study Number:

Study Director:

This study has been audited by Huntingdon Life Sciences Ltd. Quality Assurance Department (Huntingdon). The methods, practices and procedures reported herein are an accurate description of those employed at Huntingdon during the course of the study. Observations and results presented in this final report form a true and accurate representation of the raw data generated during the conduct of the study at Huntingdon.

Inspections were made by the Quality Assurance Department of various phases of the study conducted at Huntingdon and described in this report. The dates on which the inspections were made and the dates on which the findings were reported to the Study Director and to Management, Huntingdon Life Sciences Ltd. are given below.

Date of Inspection	Study Phase	Finding reported to:	
		Study Director	Management
19 October 1998	Protocol review	22 October 1998	22 October 1998
20 October 1998	Husbandry	22 October 1998	22 October 1998
20 October 1998	Weighing of animals	22 October 1998	22 October 1998
3 December 1998	Report audit	7 December 1998	7 December 1998

2 February 2000
Date

Quality Assurance Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

APPROVAL SIGNATURES

This report consists of Pages 1 through 15 including Table 1 and Appendix 1.

10 Feb 2000
Date

Management,
Huntingdon Life Sciences Ltd.

10 Feb 2000
Date

Study Director,
Department of Acute Toxicology,
Huntingdon Life Sciences Ltd.

9 February 2000
Date

Senior Study Supervisor,
Department of Acute Toxicology.
Huntingdon Life Sciences Ltd.

21 Jan. 2000
Date

Sponsor Representative.

STUDY INFORMATION

Study Initiation Date:	October 15, 1998
Experimental Start Date:	October 20, 1998
Experimental Termination Date:	November 2, 1998
Study Completion Date:	10 February 2000
Study Director:	
Sponsor:	
Sponsor Representative:	
Study Supervisor:	
Senior Technician for the study:	
Head, Department of Acute Toxicology:	
Chief Technician:	
Director Quality Practices:	
Head, Department of Analytical Chemistry and Pharmacy:	
Head, Department of Microbiology:	
Head, Veterinary Services:	

Skin Irritation of in the Rabbit

I. INTRODUCTION

The objective of this study was to assess the skin irritation potential of following a single dermal application to the rabbit.

II. MATERIALS AND METHODS

A. Test Substance : lot number BN028339, was received at Huntingdon Life Sciences Ltd. on April 6, 1998. The test substance was a pale yellow liquid, and was stored at room temperature (ambient temperature between 10 and 30°C). The Huntingdon Test Substance Data Sheet indicated that the test substance was stable until 28 February 2001. The test substance, as received, is regarded as the "pure" material and is representative of All the remaining test substance will be returned to the sponsor after the completion of all the relevant studies, with the exception of a 1 g sample which will be retained by Huntingdon Life Sciences Ltd. Test substance characterisation has been carried out by the Sponsor (Study number). As the test substance was administered as supplied, assessment of solubility was not applicable in this study. The absorption of the test substance was not quantitated.

B. Dosage Formulation: The test substance was shaken and administered undiluted.

C. Animals: New Zealand White rabbits (HsdPoc:NZW) weighing between 1928 and 2753g at receipt were obtained from Harlan UK Ltd, Shaw's Farm, Blackthorn, Bicester, Oxon, England on October 8, 1998, and kept in isolation. They were observed daily for signs of ill-health and following a review of health monitoring procedures (absence of clinical observations and satisfactory body weight gain) by a veterinary officer, three healthy rabbits were randomly selected from the stock order after 11 days of acclimatisation. All three rabbits were female. The animals were identified by a numbered aluminium tag placed through one ear on arrival. These numbers were unique within the Huntingdon Life Sciences Ltd. Acute Toxicology Department throughout the duration of the study. The cage was identified by a coloured label displaying but not limited to the study schedule number, animal number and initials of the Study Director and Home Office Licencee. Rabbits of the New Zealand White strain were chosen as the test species as they have been shown to be a suitable model for skin irritation studies and are the species recommended in the test guidelines. The rabbits were dosed by topical application as the test substance may come into contact with the skin during handling or use.

D. Food and Water: The rabbits were provided, *ad libitum*, with a standard laboratory diet, SDS Stanrab (P) SQC Rabbit Diet (supplier: Special Diet Services Ltd, Witham, Essex) and drinking water via an automatic watering system (supplier: Anglian Water). Autoclaved hay was supplied three times weekly. The batches of diet were analysed once, by the supplier, for nutrients, possible contaminants and micro-organisms, likely to be present in the diet, and which, if in excess may have an undesirable effect on the test system. Results of routine physical and chemical analyses of drinking water performed by the supplier are made available to Huntingdon Life Sciences Ltd. as quarterly summaries. Water was supplied in conformity with EC Directive 80/778/EEC and UK Water Act 1989 and subsequent amendments. No contaminants capable of adversely affecting the integrity or interpretation of the results from this study were known to be present in the basal diet or the drinking water during the conduct of this study. The Study Director reviewed the feed and water analyses. The certificates of analyses will be lodged in Huntingdon Life Sciences Ltd. Archives. Samples of water were taken from the drinking water at source in the animal room prior to the study start. The samples were analysed for microbial contaminants (total viable count, coliform count and *E.Coli* count) by Huntingdon Life Sciences Ltd. Department of Microbiology. A certificate of analysis is appended to this report.

E. Housing and Environment: The rabbits were housed individually in a suspended metal cage with perforated floor measuring 45.5 cm high, 76 cm wide and 60.5 cm deep (floor area 4598 cm^2). The cage size is in compliance with UK animal welfare guidelines. Absorbent cage liners were placed in the pan below the metal mesh floor of the animal cages to absorb liquids. During the treatment phase of the study, animal room temperature and relative humidity were continuously recorded, using a seven day recorder. Minimum and maximum parameters were noted daily and ranged from 15 to 22.5°C and 44 to 78%, respectively. Air exchange was set to provide approximately 18 air changes per hour. Fluorescent lighting was controlled by means of a time switch and provided 12 hours of artificial light (0700 - 1900 hours) which was followed by 12 hours of darkness in each 24 hour period.

F. Methods:

1. Animals: The three female rabbits, nulliparous and nonpregnant, were allocated to the study using a random numbers table. The randomised list of cage numbers 1-50 (animal numbers 1414-1463) was generated using the statistical software package Genstat version 5 Release 3.2, utilising the randomisation directive (Payne R.W *et al* 1993*Genstat 5 Release 3 Reference Manual.* Clarendon Press Oxford). Animals were in the bodyweight range 2193 to 2616 g and were at least 11 weeks of age on Day 1 of the study. The animals were acclimatised to the laboratory environment for 12 days.

2. Preparation: One day prior to application of the test substance (approximately 24 hours), hair was removed with electric clippers from the dorso-lumbar region of the rabbit taking care not to damage the skin, exposing an area of skin approximately 100 mm x 100 mm (approximately 10% of the total body surface area). The skin was not abraded.

3. Dosing: On the day of treatment (October 20, 1998), a single 0.5 ml dose of the test substance, was applied using a 2 ml plastic syringe, under a 2-ply 25 mm x 25 mm (6.25 cm^2) porous gauze pad to the previously clipped intact dorso-lumbar skin site of each rabbit. The treatment area (approximately 100 mm x 100 mm) was covered by a semi-occlusive dressing (Elastoplast elastic adhesive bandage 7.5 cm) encircled firmly around the trunk of the animal, the end of which was secured with "Sleek®". The animals were not restrained during the exposure period and were returned to their cages immediately after treatment. At the end of the 4 hours exposure period the dressings were carefully removed and the treated area of skin was washed with water (35°C) to remove any residual test substance. The treated area was blotted dry with absorbent paper.

4. Observations: The rabbits were observed twice daily for mortality and morbidity.

5. Body Weights: The rabbits were weighed on arrival, immediately prior to dosing and at sacrifice.

6. Clinical signs: The rabbits were observed daily for any signs of ill health and toxicity. Observations were made at the cageside during the twice daily standard mortality and morbidity checks and when animals were removed from the cage to determine dermal responses.

7. Dermal Responses: The treated skin of the rabbits was examined on Day 1 (approximately 60 minutes after removal of the dressings) and on Days 2, 3 and 4 (24, 48 and 72 hours after removal of the dressings), additional observations were made on Days 5 through 10 for all animals, on Day 11 for two animals and on Days 12 through 14 for one animal. At each interval, dermal irritation was assessed according to the following prescribed arbitrary numerical system (based on Draize JH, Appraisal of the Safety of Chemicals in Foods, Drugs & Cosmetics, Assoc. Food & Drug Officials of the US, Austin, TX; 1959):

Erythema and eschar formation:

No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) or eschar formation (injuries in depth) preventing erythema reading	4

Oedema formation:

No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 millimetre)	3
Severe oedema (raised more than 1 millimetre and extending beyond the area of exposure)	4

Any other lesion not covered by this scoring system was described.

The Primary Irritation Index (PII) was calculated according to the following formula (Technical Report No.66 "Skin irritation and Corrosion: Reference chemicals in data bank" (March 1995) ECETOC, Brussels):

$$PII = \frac{\sum \text{ ERYTHEMA at 24 / 48 / 72 hrs} + \sum \text{ OEDEMA at 24 / 48 / 72 hrs}}{3 \times \text{no. of animals}}$$

The maximum possible PII was 8.

8. Animal Disposition: After the final observation (October, 29, 30 or November 2, 1998) the rabbits were sacrificed by an intravenous overdose into the marginal ear vein of pentobarbitone sodium B.P. 200 mg/ml (Euthatal manufactured by Rhône Mérieux Ltd., Harlow, Essex, England) and discarded without necropsy.

G. Location of Study Records: The protocol and all raw data as well as a sample of the test substance and study related documents generated during the course of the study at Huntingdon Life Sciences Ltd., together with the original final report are lodged in the Huntingdon Life Sciences Ltd., Archive, Huntingdon, England. Such records will be retained for a minimum period of five years from the date of issue of the final report. At the end of the five year retention period the client will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the client's prior approval.

H. Statistical Analysis: None conducted.

III. RESULTS

A. Mortality: No deaths occurred during the study.

B. Body Weights: The weight for the rabbits used in this study was in the range 2193 to 2616 g at treatment initiation.

C. Clinical Signs: There were no signs of systemic reaction to treatment.

D. Dermal Responses: A single semi-occlusive application of to intact rabbit skin for four hours elicited well-defined erythema in all three animals and was accompanied in one by very slight oedema. Desquamation of the stratum corneum developed in all animals. Dermal reactions had resolved completely in all animals by Day 10, 11 or 13.

The Primary Irritation Index (PII) was calculated to be 2.11.

IV. CONCLUSION

Under the conditions of this study, elicited well-defined dermal irritation.

V. DEVIATIONS FROM PROTOCOL

There were no deviations that were considered to have affected the quality or integrity of the data from the study. However the following deviations did occur:

During the study the temperature range was recorded to be 15 to 22.5°C. These values were outside the range of 17-21°C for temperature stated in the protocol.

The higher value for humidity recorded was 78%. This exceeded the 30 - 70% tolerance stated in the protocol.

Information regarding the pH of the test substance was not available from the Sponsor prior to the start of the study. Therefore in order to comply with regulatory and UK Home Office guidelines, the pharmacy department at Huntingdon Life Sciences measured the pH of the test substance, 10%, using a pH meter. The resultant measurement was used in conjunction with other practices to enhance animal welfare. However the Sponsor has indicated that based upon the nature of the test substance the measurement of the pH was inappropriate.

Examination of the treated skin was made 56 minutes after removal of the bandage.

Contrary to Huntingdon Life Sciences protocol study number), the water from the automatic watering system exit was not sampled on this occasion. It is not considered that this omission had any effect on the scientific interpretation of the study.

There were no other deviations.

VI. TABLE 1

Dermal Reactions

Rabbit no. & sex	E = Erythema O = Oedema	Day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1450 Female	E	1	2	2	2	2	2	1a	1a	1a	1a	0a			
	O	0	0	1	1	1	1	1	1	0	0	0			
1428 Female	E	1	2	2	2a	2a	2a	1a	1a	1a	1a	1a	1a	0a	0a
	O	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1451 Female	E	1	2	2	1	1a	1a	1a	1a	1a	0				
	O	0	0	0	0	0	0	0	0	0	0				

Primary Irritation Index = 2.11.

a Desquamation (Characterised by dryness and sloughing).

VII. APPENDIX 1

Certificate of analysis for microbial contaminants of water

CERTIFICATE OF ANALYSIS

MICROBIOLOGICAL ANALYSIS OF ANIMAL DRINKING WATER

Huntingdon Life Sciences study number :		
Report number :		
Source of water sample (s):	Huntingdon Research Centre, Building R14 Room 2 (1) Cold water tap entry. (2) Automatic watering system exit*.	
Date sampled and tested :	30 September 1998	
Test procedure :	Protocol for Huntingdon Life Sciences study number study number approved 11 June 1998.	
Research Laboratory :	Huntingdon Research Centre Department of Cellular Sciences P O Box 2 Huntingdon Cambridgeshire PE18 6ES ENGLAND	
RESULTS	**Count**	**Specification**
Total viable count for aerobic bacteria :	(1) 1 cfu/ml (22°C)	$<10^4$ cfu/ml (22°C)
	(1) <1 cfu/ml (37°C)	$<10^2$ cfu/ml (37°C)
Total viable count for presumptive coliform bacteria :	(1) <1 cfu/100ml	<1 cfu/100ml
Total viable count for presumptive *E.coli* :	(1) <1 cfu/100ml	<1 cfu/100ml
CONCLUSION:	Sample (1) showed satisfactory microbiological quality.	
Protocol Deviations:	* Contrary to the protocol, the Automatic watering system exit was not sampled on this occasion.	
Results reviewed by : **Head, Microbiology**	**Signature :** **Date :** 13 May 1999	

cfu - colony forming unit